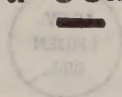


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**BREEDING FOR
IMPROVED OIL AND MEAL QUALITY
IN RAPE (*Brassica napus* L.) AND
TURNIP RAPE (*Brassica campestris* L.)**

by Roland Jönsson





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av

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- I. Appelqvist, L.-Å. and R. Jönsson, 1970. Lipids in Cruciferae. VII. Variability in erucic acid content in some high-erucic acid species and efforts to increase the content by plant breeding. *Z. Pflanzenzüchtg.* 64, 340-356.
- II. Jönsson, R., 1973 a. Breeding for low erucic acid content in summer turnip rape (*Brassica campestris* L. var. *annua* L.). *Z. Pflanzenzüchtg.* 69, 1-18.
- III. Jönsson, R., 1973 b. (Breeding for improved composition of fatty acids in oil plants. I. Decreased content of erucic acid in summer turnip rape by selection in Swedish market varieties.) *Sveriges Utsädesförenings Tidskrift* 83, 179-186. (In Swedish with English summary.)
- IV. Jönsson, R., 1974. Breeding for low erucic acid content in winter turnip rape (*Brassica campestris* L. var. *biennis* L.). *Z. Pflanzenzüchtg.* 73, 259-268.
- V. Jönsson, R., 1975 a. (Breeding for improved composition of fatty acids in oil plants. II. Investigation on the effect of some environmental factors on the fatty acid composition in winter rape.) *Sveriges Utsädesförenings Tidskrift* 85, 9-18. (In Swedish with English summary.)
- VI. Jönsson, R., 1975 b. (Breeding for improved composition of fatty acids in oil plants. III. Increased content of linoleic acid in summer turnip rape.) *Sveriges Utsädesförenings Tidskrift* 85, 19-29. (In Swedish with English summary.)
- VII. Jönsson, R., 1975 c. (Yellow-seeded rape and turnip rape. II. Breeding for improved quality of oil and meal in yellow-seeded materials.) *Sveriges Utsädesförenings Tidskrift* 85, 271-278. (In Swedish with English summary.)
- VIII. Jönsson, R., 1977. Erucic acid heredity in rapeseed (*Brassica napus* L. and *Brassica campestris* L.). *Hereditas* 86, 159-170.
- IX. Jönsson, R. and L. Bengtsson, 1970. (Yellow-seeded rape and turnip rape. I. Influence of breeding for yellow seeds upon yield and quality properties.) *Sveriges Utsädesförenings Tidskrift* 80, 149-155. (In Swedish with English summary.)
- X. Jönsson, R., E. Josefsson and B. Uppström, 1975. (Breeding for low content of glucosinolates in rape and turnip rape.) *Sveriges Utsädesförenings Tidskrift* 85, 279-290. (In Swedish with English summary.)

BREEDING FOR IMPROVED OIL AND MEAL QUALITY IN RAPE (*Brassica napus* L.) AND
TURNIP RAPE (*Brassica campestris* L.).

INTRODUCTION

Large scale cultivation of oil crops as a raw material for the manufacture of margarine started in Sweden during the second world war. Among the many species tested as oil crops during the past years, rape and turnip rape have turned out to be the ones best adapted to the prevailing climatic conditions. Winter and summer forms of these species occupy at present about 99 % of the total Swedish oil-crop acreage. In recent years this area has annually amounted to about 150,000 ha. Breeding work with rape and turnip rape started at the same time as the large scale cultivation and was, from the beginning, concentrated on improvements of seed yield, oil content and agronomic properties. Demands for improved quality of oil and meal have been added during the past two decades. At present the plant breeding work is concentrated on quality with the aim of augmenting the marketing possibilities for rape seed in the country and thereby facilitating an unaltered, or preferably increased, oil-crop acreage. This would be desirable both from the point of view of crop cultivation and emergency.

IMPROVED OIL QUALITY

The quality characteristics of an oil are determined by its fatty acids. Some data on the amounts of the most important fatty acids are given in Table 1.

Table 1. Percentage allotments of the most important fatty acids
in oils from various agricultural crops.

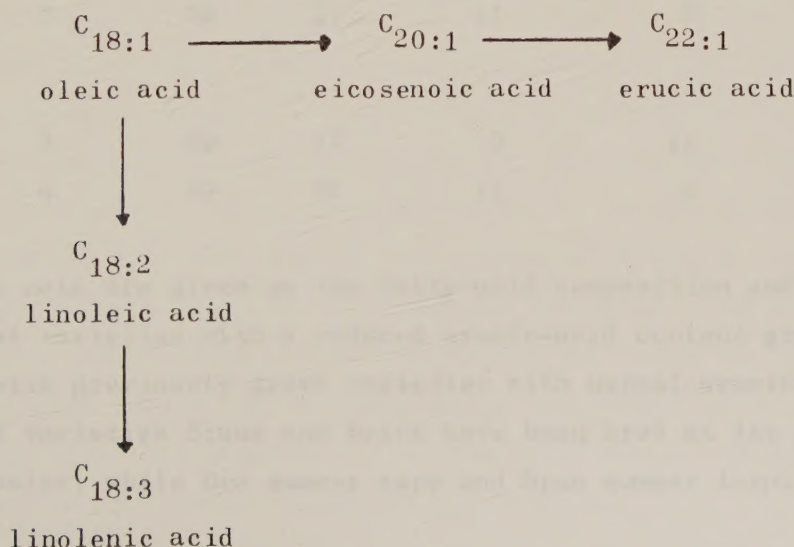
Oil from	Fatty acid					
	Palmitic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic
Winter rape	4	11	13	9	9	52
Summer rape	4	17	14	12	13	38
Winter turnip rape	3	14	14	10	11	46
Summer turnip rape	3	29	17	9	11	29
Cotton seed	22	21	54			
Groundnut	11	37	39			
Soybean	11	25	49	8		
Sunflower	7	24	65			
Poppy	11	14	73	1		

These data relate to rape and turnip-rape types that have not been subjected to selection for improved oil quality, and to some imported plant oils that are used as primary materials for margarine production. Oils of rape and turnip rape differ markedly from those of other primary margarine materials by their containing eicosenoic and erucic acid, the latter in very large amounts. These oils also contain comparatively large amounts of linolenic acid and small amounts of linoleic and palmitic acid.

Feeding experiments with animals have shown that erucic acid may cause fatty deposition and myocardial lesions (VLES 1974). Because of the risk that such deleterious effects might also appear in man the amount of erucic acid in margarine ought to be kept as low as possible. Linolenic acid is a polyunsaturated and, therefore, desirable fatty acid. It is, however, easily oxidized, resulting in taste impairment. The amount of this fatty acid should, therefore, be kept at a minimum in margarine. An increase in the amount is, however, desirable with regard to linoleic acid, a doubly unsaturated and essential fatty acid, and the same holds true for palmitic acid, which provides a firm fat and is thus of importance for the consistency of the margarine. A prerequisite for a considerable increase in the amounts of linoleic and palmitic acids in rape and turnip-rape oil is a reduction to a minimum in the amounts of the undesirable fatty acids.

The main biosynthetic pathways for the more important fatty acids in rape and turnip rape, and the number of carbon atoms and double bonds characteristic for the different fatty acids are given in Figure 1. Since each of the two

Figure 1. Biosynthetic pathways of the main fatty acids in rapeseed.



unsaturated fatty acids, erucic and linolenic acid, constitute an end product of a biosynthetic pathway; a reduction in the amount of these fatty acids can be achieved if the enzymes that control the synthesis from oleic to erucic acid and from linoleic to linolenic acid can be made less efficient or prevented from functioning.

The content of erucic acid

Materials with a block in the biosynthetic pathway towards eicosenoic and erucic acid have been found in turnip in both summer rape (BILBROOK, 1951; and DUFFET 1951) and summer turnip rape (DUFFET 1951). These materials have been utilized in the plant breeding work. As a result of this, new varieties of summer and winter rape and summer turnip rape, all with a low erucic-acid content (i.e. with less than 2 %), have replaced the varieties previously grown in our country.

Table 2. Main fatty-acid composition and crude fat yield in the first varieties with a reduced erucic-acid content cultivated in Sweden in comparison with some earlier cultivated varieties (underlined) with a normal erucic-acid content.

Group and variety	Fatty-acid composition in percent						Crude fat yield in official trials Bel.fig.
	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid	
<u>Winter rape</u>							
Wintor	4	19	13	10	9	52	<u>100</u>
Wintor	3	43	17	12	7	12	88
Bruck	3	55	22	13	2	<2	93
<u>Summer rape</u>							
Walden	4	17	14	12	13	38	<u>100</u>
Walden	3	76	21	11	2	<2	88
<u>Summer turnip rape</u>							
Turnip	3	29	17	9	11	29	<u>100</u>
Turnip	4	56	22	11	2	<2	89

In Table 2 some data are given on the fatty-acid composition and yield of crude fat of the first varieties with a reduced erucic-acid content grown in our country, in comparison with previously grown varieties with normal erucic-acid content. The winter-rape varieties Wintor and Bruck have been bred at the Swedish Seed Association, Walden while the summer rape and turnip summer turnip rape are Canadian varieties.

The reduction of erucic-acid content in rape and turnip rape to below 2 % has resulted in a drastic reduction to less than 1 % in eicosenoic-acid content.

a drastic rise to more than 55 % in the oleic-acid content, a comparatively strong rise in linoleic-acid content, to somewhat more than 20 %, and in a minor change in linolenic-acid content (JÖNSSON 1973 a, 1974).

The change-over to low erucic-acid varieties has resulted in lower yields of crude fat. This is partly due to the materials used as gene sources having very inferior agronomic properties, and that a summer-rape material was used as gene source also for winter rape. A contributing factor is also that the plant-breeding work, aiming at a reduction in erucic-acid content, only has been pursued for a comparatively short period of time. The Canadian low erucic-acid varieties of summer rape and summer turnip rape are lower yielding because they are less well adapted to our climatic conditions.

The old varieties of winter rape have been replaced by Swedish varieties with low erucic-acid content. The variety Sinus, which replaced the varieties with a normal erucic-acid content in the autumn of 1971, was a compromise with regard to reduced erucic-acid content and good cultivability. Thanks to this variety there was no drastic reduction in the Swedish winter-rape acreage. This could otherwise have been the case, since, at that time, oil-crop growing as such was questioned because of reports on the harmful effects of erucic acid. In the autumn of 1976 this variety was replaced by Brink, a low erucic-acid variety with very good winter hardiness and a yielding capacity close to that of the old varieties with a normal erucic-acid content. German and French low erucic varieties have an unsatisfactory winter hardiness under normal winter conditions even in the southern most parts of Sweden. These varieties are therefore lower yielding than the Swedish varieties.

Our breeding efforts have been less successful with regard to summer rape and summer turnip rape. The first low erucic varieties of these species to be grown in our country are of Canadian origin. The causes for this are manifold. The breeding for improved oil quality started earlier in Canada than in other countries because low erucic material was first found there. Furthermore the growing of oil-crops in Canada is concentrated on the summer forms of rape and turnip rape. The smaller resources assigned for oil-crop breeding in Sweden are split up, since breeding is also performed with the winter types of these species and with poppy, sunflower and white mustard. Quality breeding in Canada was, from the very beginning, directed towards the production of erucic-acid-free varieties, whereas intermediate varieties were also tested in Sweden. More stringent rules with regard to the homogeneity and stability of varieties, and the successively increased demands on reduced erucic-acid content, have meant that such varieties are now no longer of any interest.

Different breeding methods have been used in the production of low erucic-acid varieties. Inbreeding has been the dominant method at the Swedish Seed

Association, and is achieved through bag isolation of inflorescences at the bud stage. Oil-quality analysis is performed on oil from a number of seeds from such an isolation. The next generation is raised from other seeds from the same isolation. The half-seed method (DOWNEY and HARVEY 1963; RAKOW and THIES 1971; THIES 1971) has been used in Canada. This method implies that the analysis is made on oil from one of the cotyledons of a germinating seed, while a plant is raised from the rest of the seed.

Determination of oil quality is performed using gas chromatography (APPELQVIST 1968a, b) or paper chromatography (THIES 1971). The accuracy of the analyses is satisfactory even on oil from single cotyledons. The fatty-acid composition of the larger cotyledon agrees well with that of the whole seed (THIES 1971). The erucic-acid content of the oil is more accurately determined from an analysis of a single seed than from many seeds, among which different genotypes may be present. Paper chromatography may, therefore, well be used in half-seed analyses in determining presence or absence of erucic acid. The gas-chromatography method, used as a routine method at the Swedish Seed Association for determination of fatty-acid composition, does not completely separate all fatty acids present (JÖNSSON 1973a). Thus, the amounts of eicosenoic and erucic acid determined include also the other fatty acids with 20 and 22 carbon atoms respectively. The amounts of these last mentioned fatty acids are, however, very small and do not appreciably influence the results.

The half-seed technique furnishes the breeder with a perfect control of his material since the seed analysed gives rise to the following generation and crosses can be performed on plants with known genotypes. Variations in fatty-acid composition within the progenies from single plants can be better utilized, and extreme types found easily preserved. The speed in the breeding procedure can be considerably increased since a program, including backcrosses in each generation, can be performed (JÖNSSON 1973a), and since selection for seeds almost completely free of erucic acid can be performed on seeds from F_1 -material. The inbreeding method allows this selection to be carried out at the earliest on seeds from F_2 -plants, a selection that often has to be repeated in later generations. Selection using the half-seed technique implies that the amount of the breeding material can be considerably reduced and, thus, be grown in greenhouses. In this way the breeding work can be performed independent of season and it is possible to grow two generations per year of the summer types and more than one generation per year of winter types, if these are vernalized at $+2^{\circ}\text{C}$ for 4-5 weeks. The half-seed technique also requires less labour. Genetically stable, low erucic varieties are more easily, and with greater safety, obtained from selections based on single seeds. The risk of a rise in erucic-acid content, as a result of

outcrossing is eliminated with this method, since it is possible to avoid the possibility of intermediate material being grown together with low erucic material in the experimental fields. The half-seed technique also gives faster and more reliable data with regard to the genetics of fatty acids.

It is a well known fact that inbreeding in turnip rape results in strongly reduced fertility and vitality. Inbreeding is, therefore, a less suitable method for improvement of oil quality in this species. Inbreeding also has a strong negative effect on rape (KLOEN 1950; WAGNER 1954; MANNER 1959). These results seem to have been forgotten and methods implying very strong inbreeding have been dominating the breeding work in many countries during the past decades. Results from more recent and extensive investigations into winter rape by SCHUSTER and MICHAEL (1976) also demonstrate a strong negative inbreeding effect, particularly with regard to seed yield (Table 3). Crosses between inbred lines gave

Table 3. Inbreeding effects in winter rape (from Schuster and Michael 1976).

I-and F- generations	No. of lines tested	Seed yield		Oil content in dry matter		Plant height	
		No. of lines	Rel. fig.	No. of lines	Rel. fig.	No. of lines	Rel. fig.
I ₀	380		100		100		100
I ₁	1549		69		98		93
I ₂	388		66		101		92
I ₃	187		61		97		93
I ₄	97		54		98		92
I ₅	45		49		92		94
I ₆	65		51		96		93
I ₇	77		52		91		108
I ₈	17		45		-		94
I ₀		580	100	580	100	580	100
I ₁ - I ₈		2425	56	2115	92	1138	95
F ₁		846	91	846	92	258	94

heterosis effects but the original yield level was not restored. Therefore inbreeding can be successfully applied in the breeding work only if a breeding program is followed that considers the need to restore vitality. Such programs are very time-consuming and are thus not applicable in the present situation, which calls for a rapid turn-over of varieties with gradually improved quality and cultivability. When the half-seed technique is applied there is no need for

a forced inbreeding with bag isolation.

During the 1970's the half-seed technique has also been used to an increasing extent at the Swedish Seed Association. Analyses of individual seeds of Swedish marketed varieties of rape and turnip rape have demonstrated the presence of a very large variation in erucic-acid content. Almost completely erucic-acid-free seeds were found after three generations of selection in the summer turnip-rape varieties Bele and Torpe (JÖNSSON 1973a, b). Selections from both these varieties are characterized by a considerably better stalk stiffness than the Canadian low erucic material (JÖNSSON 1973b). A large number of low erucic lines, originating from these selections, are at present being tested in comparative yield trials. Two of these low erucic selections from Torpe, displaying as high a crude fat yield as that of the mother variety, are being tested in official trials in 1977. Almost completely erucic-acid-free seeds have also been obtained through repeated selections in summer-rape varieties with normal erucic-acid content. The frequency of such plants is, however, much too small for a direct selection to be successfully applied in the breeding work with this species. No seeds, completely lacking erucic-acid genes have been found in marketed varieties of winter turnip rape, in spite of repeated selection for many generations (JÖNSSON 1974).

The half-seed technique has been applied in backcross programs in order to transfer genes preventing the formation of erucic acid, to high yielding and cultivable varieties. The above mentioned low erucic-acid material of summer rape found in Canada has been used as a gene source for summer and winter rape. The corresponding sources for summer turnip rape have been a Canadian summer turnip rape, low in erucic acid, and low erucic-acid selections from Torpe and Bele. This last-mentioned material has been used also as gene source for the production of low erucic-acid winter turnip rape (JÖNSSON 1974). The varieties, mainly used as recurrent parents, have been Norde winter rape, Rapido III winter turnip rape, Gulle summer rape and Torpe summer turnip rape. One such backcross program, that started in the spring of 1971, resulted in the autumn of 1976 in the approval of Gulliver summer rape from the cross Gulle x (Gulle x Selection from Hermes) as a market variety. Gulliver and Olga, a new high yielding low erucic variety from Weibullsholm, in 1977 replaced the Canadian variety Oro over the main part of the Swedish summer-rape acreage. Gulliver is almost equivalent to the recurrent parent Gulle with regard to yield and other more important agronomic characters (Table 4). This variety is a good illustration of the possibility of speeding up the breeding process through a combination of backcross and half-seed techniques. A number of other promising breeding lines of

Table 4. Comparison between the low erucic variety Gulliver summer rape and its recurrent parent Gulle. Figures given are average figures from 50 comparative yield trials during the years 1973-1976.

Characters	Gulle	Gulliver
Seed yield, rel.	100	102
Oil content in dry matter, %	44.7	43.9
Crude-fat yield, rel.	100	100
Maturity, differences in days		-1.8
Stalk stiffness (1-100)	84	85
Erucic-acid content, %	38	42

summer rape and summer and winter turnip rape, originating from such backcross programs which started in 1971 or later, are at present being tested in official trials. However, further backcrosses are needed in order to improve the winter hardiness of the winter turnip-rape material.

The experiences from the breeding of low erucic-acid varieties have yielded a gradually improved knowledge of the genetics of erucic-acid content. According to DOWNEY and CRAIG (1964) the erucic-acid content in summer rape is controlled by two additively working genes without dominance. Each allele is reported to increase the content with 9-10 %-units (HARVEY and DOWNEY 1964). According to KONDRA and STEFANSSON (1965) the same genes also control the eicosenoic-acid content, in this case exhibiting dominant effects. The erucic-acid content in summer turnip rape is controlled by one gene (DORRELL and DOWNEY 1964). This concept of the genetics of erucic-acid content, which is presented also in quite recent publications (LEE et al. 1974; MORICE 1974), does not give adequate account for the presence of stable erucic-acid levels at about 10 % in Bronowski summer rape or at about 60 % in winter rape and summer turnip rape (APPELQVIST and JÖNSSON 1970). Nor is this concept capable of explaining the often rather indistinct segregation ratios found in the F_2 (HARVEY and DOWNEY 1964; LAMMERINK and MORICE 1971; DORRELL and DOWNEY 1964). The presence of alleles that control a more or less effective erucic-acid production has also been reported repeatedly (STEFANSSON and HOUGEN 1964; KRZYMANSKI and DOWNEY 1969; LAMMERINK and MORICE 1971).

The selection work with the help of the half-seed technique has revealed the presence of series of multiple alleles that control the production of erucic acid. A large number of homozygosity levels have been discovered. Such levels within the regions 5-10, 10-35 and above 35 % erucic acid are controlled by alleles at one, one or two and two loci respectively. The least effective allele,

yielding less than 2 % erucic acid, can be distinguished from the one, yielding only traces of erucic acid; since the former allele also controls the production of more than 8 % eicosenoic acid (JÖNSSON 1977). Most probably, this allele controls the synthesis from eicosenoic to erucic acid, while the allele, yielding low amounts of eicosenoic and erucic acid, is controlling the synthetic step from oleic to eicosenoic acid. The erucic-acid content in turnip rape is controlled by multiple alleles at one locus, as demonstrated by the results of backcross programs in which the half-seed technique has been utilized (JÖNSSON 1973a, 1974). The presence of multiple alleles implies a risk for misclassification during the realization of a backcross program using the half-seed technique. However, this problem can be mastered, if varieties with known allele combinations are used as recurrent parents and if the content of eicosenoic acid is taken into consideration too.

The correlation between amounts of erucic and eicosenoic acid is positive at levels below 25 % erucic acid, but negative at higher levels. The alleles exhibit additive effect for erucic acid at levels below 30 % while partial dominance is common at higher levels. Those alleles, that control low amounts of erucic acid exhibit partial dominance with regard to eicosenoic-acid content. Alleles that control a more effective erucic-acid production, on the other hand, exhibit strong overdominance with regard to the eicosenoic-acid content (JÖNSSON 1977).

High linoleic-acid content

Removal of erucic acid has resulted in an increase in linoleic-acid content from approximately 13 to 21 %. Considerably higher linoleic-acid contents are, however, desirable in rape and turnip-rape oils, in order to enhance their usefulness as raw materials for the manufacture of margarine. Gaschromatographic analyses have revealed linoleic-acid contents of up to about 50 % in breeding material of summer rape. The linoleic-acid content, however, is considerably influenced by environment, in contrast to the erucic-acid content (RÖBBELEN and THIES 1973) and such extremely high contents were not recovered in the following generations. Growth-chamber experiments have been performed with inbred, low erucic winter-rape plants in order to gain more knowledge regarding environmental influences on the fatty-acid pattern. Lower contents of palmitic and linoleic acids and a higher content of oleic acid was obtained at a temperature of +21°C when compared to +14°C. The same linolenic-acid content was found at both temperatures. An increase in day length from 14 up to 24 hours had no effect on palmitic-acid content. However, the content of oleic acid was increased, those of linoleic and linolenic acids were reduced, and the quotient between these two

last-mentioned acids was larger. The highest linoleic-acid content was obtained under short day conditions and low temperatures while the lowest linolenic-acid content was obtained under long day conditions and was unaffected by temperature (JÖNSSON 1975a). These results agree fairly well with those of CANVIN's (1965). APPELQVIST (1971) found, however, that the content of oleic acid in summer rape was reduced at higher temperatures. One explanation of this contradicting result might be that the temperature within a 24 hours day in this experiment varied within a range of 12 centigrades, whereas the temperature was constant in the other experiments. Nor can the existence of genetic differences with regard to the reaction to varying environments be excluded. APPELQVIST (1971) also concluded that long day conditions resulted in lower linolenic-acid contents than short day conditions. Light-intensity variations between 7.800 and 13.900 lux did not substantially influence the fatty-acid pattern in winter rape (JÖNSSON 1975a).

The growth-chamber experiments carried out so far have not been able to explain in a satisfactory way the occurrence and instability of extremely high linoleic-acid contents. However, breeding work with summer turnip rape, aiming at an enhancement of the linoleic-acid content, has been successful. A large variation in linoleic-acid content was found both between and within populations with a low erucic-acid content. Through crosses between plants with high linoleic-acid contents it has been possible to increase this content in one step in 5-6 %-units. Stable levels at above 30 % have been achieved as a result of selection with the aid of the half-seed technique. The heritability with regard to the linoleic-acid content was calculated to 0.45 and 0.76 respectively for selected material from the varieties Bele and Torpe when grown under greenhouse conditions. There is still a considerable variation in linoleic-acid content between individual seeds, and continued selection is expected to result in further improvements (JÖNSSON 1975b). Breeding materials of summer turnip rape and summer and winter rape, exhibiting about 30 % linoleic acid, are being tested in yield trials in 1977.

The half-seed technique enhances the possibilities to demonstrate and utilize seeds with high linoleic-acid contents. Since the space needed for the breeding material can be considerably reduced, thanks to this method, the breeding material can be grown in greenhouses under better controlled conditions than in the field. A greater safety is hereby achieved in the selection work. An analytical method, giving a complete separation between the oleic, linoleic and linolenic acids, is another important prerequisite for a good selection effect.

Possible improvements with regard to the contents of linolenic, palmitic and oleic acids

A positive correlation between the amounts of linoleic and linolenic acid has been frequently observed in rape and turnip rape (THIES 1969; SCHUSTER 1970; RÖBBELEN and THIES 1973). Negative correlations have, however, occasionally been obtained (JÖNSSON 1975b). A selection for high linoleic-acid content in the summer turnip-rape variety Torpe had no effect on the linolenic-acid content, whereas a similar selection from the variety Bele resulted in an increase in the linolenic-acid content. Selection for high linoleic-acid content and a large quotient between the amounts of linoleic and linolenic acid in material from Bele has given positive effects resulting in a reduction in linolenic-acid content (JÖNSSON 1975b). Most probably the synthesis of linoleic and linolenic acid in summer turnip rape is controlled by two independently working enzyme systems. RAKOW (1973) obtained mutants with low and high linolenic-acid contents respectively after treatment with ethylmetansulphonate. The linoleic-acid content was, however, unchanged in both mutants. This indicates that the two synthetic steps are controlled independently of each other. Thus, in the foreseeable future it should be possible to reduce the linolenic-acid content in rape and turnip rape oil to below 5 %. Breeding works with this aim are in progress i.a. in Canada, Germany and France. A reduced linolenic-acid content is also the ultimate goal for the breeding work in our country. However, the breeding work carried out so far has given priority to an increase in linoleic-acid content to 40 % with an unchanged linolenic-acid content. An increase in the palmitic-acid content to approximately 10 %, and the production of varieties yielding oil with a high oleic-acid content, around 70 %, suitable for cooking oils are likely breeding goals in the future. The prospects of reaching these goals are good since a considerable variation is present in both rape and turnip rape with regard to the amounts of these fatty acids.

High erucic-acid content

Oils with a high erucic-acid content are desirable for certain technical purposes e.g. as lubricants and as raw materials for the manufacture of plastic and perfume etc. A shortage of high erucic oils may be expected to arise in the near future as a result of the change-over to low erucic varieties of rape and turnip rape for edible fat purposes. The future domestic need of such oils must then be provided for either by the growing of other species or of special high erucic varieties of rape and turnip rape. APPELQVIST and JÖNSSON (1970) studied the variation within some species and the possibilities to increase the contents through selection. The highest amounts, somewhat above 60 %, were found in summer turnip rape, winter rape and crambe, Crambe abyssinica L. Selection efforts

in these species did not yield any positive effects. However, the amount could be raised from about 26 to 55 % in white mustard, Sinapis alba L. In this investigation the analysis of oil quality were performed on oil from a number of seeds. A further increase of some per cent in the erucic-acid content may possibly be obtained if the more effective half-seed technique is applied.

Crambe has probably no future as a producer of high erucic oils in our country because of its low seed yield and pronounced tendency to shattering. Winter rape is clearly superior to other species from a yielding point of view. However, a simultaneous growing of both low and high erucic varieties of the same species implies risks of contamination both in the field and in the store house. This problem can be solved by growing the two types in different areas. White mustard is of advantage in this connection, because this species does not cross with the Brassica species, and can be morphologically easily distinguished from them both in the plant and the seed stage. The prospects of improving meal quality in winter rape are good, while white mustard seems to be devoid of corresponding genes. High erucic-acid material will, in any case be cultivated on a comparatively small acreage. If high erucic white mustard is grown, then the comparatively small quantities of meal produced could probably be improved by technical means.

Low chlorophyll content

A high chlorophyll content in seeds of rape and turnip rape causes discolouration of the oil, which has to be removed by technical processes. The chlorophyll content in the seed is strongly influenced by cultivation techniques but is also partly a varietal character. The plant-breeding efforts are directed towards the production of varieties with even and early ripening, good stalk stiffness, winter hardiness and resistance to various parasites. With such varieties at hand there are good possibilities for the growers to produce a seed with a low chlorophyll content.

IMPROVED MEAL QUALITY

Low content of glucosinolates

When oil is extracted from seed of rape and turnip rape a meal remains with a protein content of 40-45 % of its dry matter. This protein exhibits an amino-acid composition better balanced than most other plant proteins. However, the meal cannot be used to its full value because of the presence of glucosinolates (JOSEFSSON and MUHLENBERG 1968). These are sulphur containing compounds, which yield toxic cleavage products, isothiocyanates, oxazolidinethiones and nitriles, under the influence of the enzyme myrosinase (JÖNSSON, JOSEFSSON and UPPSTRÖM 1975).

These toxic compounds cause reduced appetite and productivity, even when animals are fed with comparatively small amounts of rape meal. The nutritional value of the meal is improved if the myrosinase is inactivated by heat treatment. Glucosinolates can, however, still exert a noxious influence if they are hydrolyzed by enzymes produced by microorganisms in the digestive system of the animals, or if myrosinase is being supplied by other feed-stuffs. Glucosinolates can be removed from the meal by technical methods. These are, however, expensive and cause loss of protein and deterioration of the protein quality or both.

There are several methods to estimate glucosinolate content; the choice of method to be used depends on the characteristics of the material and the purpose of selection. If only a few number of plants in a population are expected to possess a low glucosinolate content in the meal, a preselection is performed using a simple paper test (LEIN 1970). Plants with a low content are then analysed with a microvariant of a spectrophotometric method (APPELQVIST and JOSEFSSON 1967). When more detailed information about the contents of different glucosinolates was needed the analyses were earlier made according to a method by YOUNG and WETTER (1967). Since 1974 these analyses have been performed with a gas-chromatographic method (UNDERHILL and KIRKLAND 1971; PERSSON 1974), which gives the most reliable results when analysing low contents.

Glucosinolate contents in meal from varieties of rape and turnip rape grown in Sweden vary between 3 and 8 % (JOSEFSSON and APPELQVIST 1968; JÖNSSON, JOSEFSSON and UPPSTRÖM 1975). The variation present is mainly genetically determined. The discovery that meal from the Polish summer-rape variety Bronowski only possessed about 0.5 % glucosinolates (JOSEFSSON and APPELQVIST 1968) created the necessary conditions for a reduction of glucosinolate content through plant breeding. Breeding lines, containing only traces of glucosinolates, were produced by selection in this variety. Materials with low glucosinolate contents have also been found in summer turnip rape (DOWNEY, CRAIG and YOUNG 1969; LEIN 1970).

Knowledge of the genetic background regarding glucosinolate contents has been obtained from analyses of meal from F_1 - and F_2 -plants following crosses between Bronowski and varieties with high glucosinolate contents. This content is determined by the genotype of the mother plant and selection can, therefore, be performed first in the F_2 . The presence of the glucosinolates gluconapine, progoitrine and glucobrassicinapine is controlled in rape by three gene systems dependent upon each other with dominant or partial dominant genes in three, four and four or five loci respectively (KONDRA and STEFANSSON 1970). This implies that only about 1 % of the F_2 -plants possess as low or lower contents than Bronowski. The contents of glucosinolates and erucic acid are inherited independent of each other (JOSEFSSON and JÖNSSON 1969; LEIN 1972).

Bronowski is not cultivable because of its low yielding capacity. Selected material from this variety has, however, been used as gene source for the production of low glucosinolate material in both summer and winter rape and winter turnip rape. Canadian low glucosinolate material of summer turnip rape and a breeding line of *Brassica campestris* L. ssp. *chinensis*, which has a comparatively low glucosinolate content, have been used as gene sources in the breeding of summer turnip rape. In order to improve the yielding level of the low glucosinolate material the breeding has been performed partly through crosses and repeated selection and partly with the backcross technique. High yielding market varieties with normal amounts of glucosinolates and erucic acid have been used as crossing parents in this breeding work. Winter turnip-rape material with a low glucosinolate content has been obtained from crosses between Bronowski and winter turnip rape. Plants with low glucosinolate contents and the characteristics of turnip rape were selected for in the F_2 and the F_3 . The selected plants were then backcrossed to winter turnip rape for a new cycle of selection (JÖNSSON, JOSEFSSON and UPPSTRÖM 1975).

The backcrosses should preferably be performed in the F_2 in order to speed up the breeding process. Two methods have been tested. One method implies that the glucosinolate content is determined on meal from unripe seeds. Plants with a low content are then cut down and backcrosses performed on the subsequently developed inflorescences. A safer method is to perform backcrosses on a large number of plants, determine the content on ripe seeds from these plants and utilize only seed from crosses with plants having low glucosinolate content in the further breeding work (JÖNSSON, JOSEFSSON and UPPSTRÖM 1975).

The breeding efforts with summer rape and summer turnip rape have resulted in low glucosinolate lines, which even surpass the market varieties Gulle and Torpe in seed yield. However, concerning erucic-acid content these lines were intermediate between the parents. Further backcrosses are needed in order to improve winter hardiness in winter-rape material with low glucosinolate content. Winter turnip-rape material with low glucosinolate content has not yet been tested in field trials.

The feeding value of meal from rape and turnip rape is considerably improved as a result of the reduction in glucosinolate content to extremely low levels. This improved meal may in the future replace imported protein-rich feed-stuffs. Low glucosinolate rape-meal will, probably within a short time be exploited as a raw material for the manufacture of protein-rich products intended for both animal and human consumption. An industrial method for such a manufacturing has already been developed (ANJOU et al. 1975). The prospects are good for further improvements of rape-meal quality since there is a

considerable variation with regard to protein content both in rape and turnip rape. This variation is at least partly genetically determined. Selection, performed in summer rape, has yielded positive results (JOSEFSSON and BENGTTSSON 1974). Rape protein has a more favourable amino-acid composition than soya protein (CLANDININ, SLINGER and BELL 1972). There is a genetic variation with regard to this character (GODING, DOWNEY and FINLAYSON 1972) and it should therefore be possible in the future to further improve the protein quality.

Yellow seed coat colour

Black and dark brown seed-coat colour in rape and turnip rape respectively was earlier a criterion of high seed quality. All varieties of the species, grown in Europe, exhibited this dark colour. Yellow-seeded forms are, however, present in South and East Asiatic subspecies of turnip rape. A few, occasional seeds with light seed-coat colour were found at the Swedish Seed Association in 1961 in a cross population of summer turnip rape, which also contained a line of Brassica campestris L. ssp. chinensis. This breeding line exhibited brown seed colour. Most probably, however, there were also genes for yellow seed colour present. Repeated inbreeding and selection for yellow seed coat resulted in a yellow-seeded variety. The seed yield was considerably lower than that of Bele, the oil content, however, improved by 3 %-units. Crosses with high yielding dark-seeded varieties were performed in order to improve the vitality of the yellow-seeded material. Continued breeding work has now resulted in summer turnip-rape varieties with high frequencies of yellow seed and with higher crude fat yields than those of Bele and Torpe. Seeds with light seed coat have been found also in winter turnip rape and summer and winter rape. However, it has hitherto only been possible to produce lines with partially yellow seeds in rape (JÖNSSON and BENGTTSSON 1970; JÖNSSON 1975c).

The seed-coat colour in rape and turnip rape is controlled by genes with pleiotropic effects resulting in a considerably improved oil content and reduced contents of seed coat and fibre as compared to dark-coloured seeds (JÖNSSON and BENGTTSSON 1970). Yellow seeds possess a thinner seed coat with smaller cell size than that of dark seeds. The higher oil content is mainly a result of the lower seed-coat content, partly of the fact that the oil content in the embryo is somewhat higher (STRINGAM, MCGREGOR and PAWLOWSKI 1971).

According to AHMED and ZUBERI (1971) seed-coat colour in Brassica campestris L. var. 'Toria' is controlled by one gene governing dark seed colour and dominating over yellow. The genetics of this character appears, however, to be more complicated in the summer turnip-rape material, studied at the Swedish Seed

Association. Crosses with homozygous yellow- or dark-seeded mother plants yielded completely yellow or completely dark seeds respectively, independent of the seed-coat colour of the father plant. This would indicate that the seed colour is controlled by the mother plant. However, this is contradicted by the finding of colour variation from yellow to black amongst seed from individual plants, often with an uneven distribution of the colour over the seed-coat surface. Results obtained so far indicate that the seed-coat colour in summer turnip rape is controlled by at least three genes and that dark seed colour is not completely dominant (JÖNSSON 1975c). Rape is an allopolyploid between turnip rape and cabbage, Brassica oleracea L. This is the reason for the difficulties met with in the efforts to attain genetically stable, completely yellow seeds in this species. Breeding work is under way aiming at the production of a completely yellow-seeded rape from crosses between yellow-seeded materials of turnip rape and cabbage.

A change to yellow-seeded varieties entails many advantages for the growing of these oil crops. It is much easier to determine the degree of ripening in yellow seed as the occurrence of chlorophyll is not masked by the dark seed coat. This reduces the risk for price reductions because of too high chlorophyll content as a result of premature harvest. The higher oil content gives the grower a higher seed price. Seeds with a yellow coat colour exhibit better meal quality with a higher energy content because of a lower fibre content. Discolouring of oil and lecithin is avoided and light-coloured endproducts are obtained from extractions of protein from the rape meal even without dehulling the seed. The fear that the thinner coat of yellow seeds should imply an increased frequency of dehulled and broken seeds after threshing has turned out to be unjustified.

IMPROVED OIL AND MEAL QUALITY

The breeding goal is to produce varieties with low amounts of both glucosinolate and erucic acid, so called double low varieties, and combine this with a high yielding capacity. Three methods have been applied to achieve this goal.

The combination of low glucosinolate content with low erucic-acid content can be obtained through direct selection in the progeny from crosses with Bronowski, since the erucic-acid content in this variety is only about 10 % and genes blocking the synthesis of erucic acid are present in that variety (JÖNSSON 1977). The frequency of seeds, free from erucic acid is, however, extremely low. The selection work may therefore end up with material exhibiting comparatively low erucic-acid levels in which further reduction through selection is impossible.

Crosses are made between populations exhibiting low glucosinolate content and low erucic-acid content respectively. Plants with low contents of both these compounds and selected from the F_2 -populations of the above crosses are then crossed in the F_3 with a high yielding variety. Selection and crossing is then repeated in the following F_2 and F_3 -generations respectively. The method implies that very large F_2 -populations have to be analysed with regard to the contents of the two compounds, since the desired qualities are controlled by a large number of genes. Backcrosses can therefore be performed at the earliest in the F_3 .

The third method used starts with two series of backcrosses, by which material with low glucosinolate content and low erucic-acid content respectively and both with high yielding capacities are produced. These two populations are then crossed with each other when both are considered acceptable from the point of view of yielding capacity and other agronomic characters. An erucic-acid-free F_2 -population is produced by selection of seed from F_1 -plants using the half-seed technique. Plants with low glucosinolate content are then selected from this F_2 -population. The two basic populations are successively improved and crosses between them can be repeated whenever wished. This method demands fewer analyses than the two earlier mentioned ones. The method further implies a faster breeding rate since the backcrosses can be performed in every and each second generation in the material with low erucic-acid and low glucosinolate content respectively. The breeding material can also, thanks to the half-seed technique, be limited in amount and thus be grown in greenhouses with two generations per year. This also considerable reduces the risk of unwanted crosses with other materials.

Genes for desirable quality properties have often been found in materials with a considerably inferior yielding capacity and cultivability than the ordinary cultivated material. In such a situation the backcross technique is without doubt superior to other breeding methods for a rapid transfer of quality genes to high yielding and cultivable varieties. It is true that selection in the cross Bronowski x Gulle has yielded varieties with a low glucosinolate content and as high a yielding capacity as that of Gulle, but a very large number of analyses, extensive field trials and repeated selections during many generations were required to achieve this result. Five backcrosses are theoretically required in order to transfer 98 % of the genes from one variety to another. However, in the practical breeding work, it has often been possible to achieve excellent results after only a few backcrosses. The summer rape variety Gulliver is an example of this. In this material yield and other important agronomic characters were level with the recurrent parent after only one backcross. The same yielding level

as that of the recurrent parent has also been achieved in some instances after only one or a few backcrosses in summer turnip rape. The explanation for this would be heterosis effects and that a considerable variation exists within varieties of cross fertilizers. A very high degree of heterozygosity can be maintained even if a more advanced series of backcrosses has to be performed in order to improve yield and cultivability. This is then achieved by a change at times of the recurrent parent. High yielding varieties, resistant to important diseases, are preferably used as recurrent parents. Resistance and quality characters can then be combined without the need for test and selection for resistance to be performed in every generation. Breeding lines possessing fair resistance to the fungal parasites Phoma lingam and Verticillium dahliae, have been obtained as a result of backcrosses to winter-rape varieties with a good resistance to these parasites (JÖNSSON 1974).

The first two double low summer-rape varieties Erglu and Tower, marketed in Germany and Canada respectively, exhibit too low a yielding capacity under our conditions. Swedish double low summer-rape varieties with considerably better yielding capacity have been tested in official trials since 1976. Promising double low varieties of winter rape and summer turnip rape are also being tested in yield trials. The winter hardiness of the corresponding winter-rape materials is not yet quite sufficient, and further backcrosses to winter-hardy rape varieties have to be performed.

The breeding work aiming at a combination of yellow seeds with other desirable quality characters in oil and meal is most advanced in summer turnip rape. A yellow-seeded low erucic-acid material of summer turnip rape, with a distinctly better crude fat yield than the dark-seeded low erucic variety Span, is now available (JÖNSSON 1975c). Yellow-seeded summer turnip-rape material, exhibiting very low contents of both erucic acid and glucosinolates, are being tested in yield trials in 1977. Moreover, several breeding lines of this material are characterized by a high linoleic-acid content, around 30 %.

The improvement in meal quality through removal of glucosinolates and change to yellow-seeded varieties has considerably improved the market potential for rape and turnip rape meal, both as a feed-stuff and as a raw material for the manufacture of protein-rich products. However, the market potentials with regard to the oil will not improve correspondingly even if the present breeding goals are achieved. An increased cultivation of rape and turnip rape should, therefore, result in a surplus of oil, that could not be disposed of in the country. This surplus might be reduced through less effective extraction of oil from rape and turnip rape seed. Hereby, an improvement partly of the oil quality

and partly of the energy content of the meal could be achieved. Other possible solutions to this problem would be to exploit crushed rape and turnip rape seed as feed-stuff without a pre-extraction of oil from this, or to produce special varieties, yielding a higher meal production at the expense of the oil content.

Breeding for quality in rape and turnip rape implies very drastic changes in the metabolism of these varieties. Such changes in well balanced organisms may result in deterioration of vitality and fertility. The reduced stalk stiffness, winter hardiness and yielding ability, characteristic of the low erucic varieties compared to earlier cultivated material, have, therefore, often been interpreted as results of the removal of a fatty acid necessary for the normal functioning of the plant. The good winter hardiness, typical of the low erucic winter rape variety Brink, and the good yielding capacity and stalk stiffness, characteristic of the low erucic summer rape Gulle, demonstrates, however, that erucic acid is not indispensable for the normal growth of the plant. The inferiority of the first low erucic-acid varieties, when compared to normal erucic-acid varieties, was instead a result of the gene sources for low erucic-acid content being inferior with regard to the characters mentioned. High linoleic-acid content is also considered to cause disturbances in the metabolism of rape and turnip rape. There is, however, no evidence so far in favour of this assumption. The first high linoleic material obtained in summer rape, exhibited a very weak stalk and a bad vitality. Considerably better high linoleic lines have later on been obtained both in rape and turnip rape and there are no signs of a negative correlation between high linoleic-acid content and good stalk stiffness or good general vitality. The shortcomings, characteristic of many high linoleic-acid materials, are probably results of the breeding methods used which have implied very strong inbreeding.

Glucosinolates are probably end products in the metabolism, of no significance for the vitality of the plants. They may, however, constitute a protection for the species, since their strong taste and smell makes the seed less tasty for certain animals. Some birds prefer seeds with a low glucosinolate content. Bird damage in field trials and larger multiplications with low glucosinolate material has, however, according to present experiences, been of no significance. There are theories, claiming that glucosinolates should exert a positive influence on the plants resistance to fungi and insects. However, no deterioration in this respect, caused by reduced glucosinolate content in rape and turnip rape, has hitherto been demonstrated.

Less cultivable varieties will be placed at the growers disposal during a

transition period because of the demands from the industry for a rapid change to varieties with gradually improved quality characters. As was the case at the turn-over to varieties with reduced erucic-acid contents, the growers will, most probably, receive compensation for the lower yield through a higher seed price also with regard to the other quality characters. Present experience indicates, however, that the breeding projects under way, aiming at an improvement of the quality of both oil and meal, can be realized without any loss, in the long run, in yielding capacity or cultivability. A considerable variation exists in rape, turnip rape and closely related species, with regard to a number of quality characters, which so far have hardly been subject to any breeding efforts. Therefore, the prospects are good for further improvements of both oil and meal from rape and turnip rape.

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